

Individual variation in the extent of milk fat depression in dairy ewes: rumen fermentation and biohydrogenation of fatty acids

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Take home message Individual variation in the severity of milk fat depression cannot be explained by a single mechanism (e.g., changes in rumen VFA or biohydrogenation metabolites) but most probably by a complex combination of factors.

Introduction Dairy ewes fed diets supplemented with marine lipids show large individual variations in the extent of milk fat depression (MFD) but reasons behind this variability are still uncertain. In a previous study (Frutos *et al.*, 2017), we were not able to demonstrate that differences in the milk concentration of some fatty acids (FA), particularly antilipogenic FA, and in transcript abundances of candidate genes involved in mammary lipogenesis explained individual variations in fish oil-induced MFD severity. It is known that MFD is related to active biohydrogenation (BH) intermediates that are produced under certain feeding conditions that alter rumen function (Bauman and Griinari, 2001). Therefore, we hypothesized that differences in the processes of ruminal fermentation and BH of unsaturated FA would account for the individual response in the susceptibility to the low-fat milk syndrome.

Materials & methods We used 15 lactating Assaf ewes fed a total mixed ration supplemented with 0 (control; n=5) or 20 g of fish oil/kg DM [10 animals were selected out of 22 receiving this supplemented diet and were divided in two groups: ewes showing a strong MFD (RESPON+, n=5) or a slight MFD (RESPON-, n=5); see Frutos *et al.* (2017) for details]. After 36 days on the diets, samples of rumen fluid were collected through stomach tube for pH, ammonia and volatile FA (VFA) concentrations, and for lipid analysis. Ammonia was determined by colorimetry, and VFA and biohydrogenation intermediates by gas chromatography. Data were analysed with the MIXED procedure of SAS 9.4 using orthogonal contrasts [namely, Control vs. (RESPON- and RESPON+), and RESPON- vs. RESPON+].

Results & discussion After 5 weeks on diets, the decrease in milk fat content, compared with the control, averaged 7.7% in RESPON- and 25.4% in RESPON+. Feeding marine lipids affected all rumen fermentation parameters (pH, ammonia and VFA) but only changes in VFA concentrations were linked to MFD severity ($P \leq 0.05$). Thus, the total VFA content (in mmol/L) fell from 117 in the control to 107 in RESPON- and 92 in RESPON+. Similar decreases were observed in acetate (74 vs. 68 vs. 58 mmol/L) and propionate (23 vs. 21 vs. 18 mmol/L) contents for control, RESPON- and RESPON+, respectively, while butyrate concentrations showed comparable reductions in both supplemented groups and molar proportions remained stable. A role of VFA on MFD has been consistently dismissed (see review by Bauman and Griinari, 2001). In fact, despite acetate is the main substrate for *de novo* synthesis of FA in dairy ruminants, it was widely accepted that its supply did not affect milk fat yield. Nevertheless, this has been recently challenged by Urrutia and Harvatine (2017) and would merit further research. Concerning BH metabolites, dietary fish oil had a strong effect on 18:0, with ruminal concentration in animals displaying MFD averaging only 15% of the control value ($P < 0.001$) but without significant variation between RESPON- and RESPON+. Only few minor FA (e.g., *cis*-6+7 16:1 or 17:0 *anteiso*) differed between these treatments ($P < 0.05$). Most *cis* and *trans* 18:1 isomers (e.g., *cis*-9 or *trans*-11 18:1) were favoured in rumen digesta from supplemented ewes, but no differences were found in relation to variability in responsiveness to MFD-inducing marine lipids. As expected, most demonstrated or putative antilipogenic FA (e.g., *cis*-9 16:1, 10-oxo-18:0, *cis*-11 18:1, *trans*-10 *cis*-12 CLA, *trans*-10 *cis*-15 18:2, which coeluted with *trans*-11 *cis*-15 18:2, or 22:6n-3) showed a higher concentration in ewes fed the fish oil-supplemented diet ($P < 0.05$). On the contrary, surprisingly, none of them differed significantly in relation to MFD intensity (i.e., between RESPON- and RESPON+). These results agree with those observed previously about changes in milk FA profile and mRNA abundances of lipogenic genes (Frutos *et al.*, 2017) and suggest that most probably the individual variability in the extent of diet-induced MFD will rely on a complex combination of factors.

Conclusion Rumen concentrations of VFA that are precursors of milk FA, mainly acetate, show lower values in animals with a strong MFD (i.e., in RESPON+). Quite the opposite, changes in rumen BH metabolites show very little variation in ewes displaying different degrees of the syndrome, which would rule out their role as main responsible for MFD severity.

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References

- Bauman DE and Griinari JM 2001. *Livestock Production Science* 70, 15-29.
- Frutos P, Toral PG and Hervás G 2017. *Journal of Dairy Science* 100, 9611-9622.
- Urrutia N and Harvatine KJ 2017. *The Journal of Nutrition*. 147 763-769.

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**Herbivore nutrition supporting sustainable intensification
and agro-ecological approaches**

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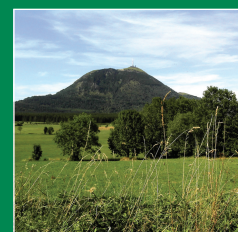
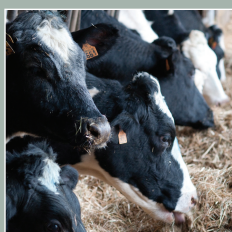
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INDIVIDUAL VARIATION IN THE EXTENT OF MILK FAT DEPRESSION IN DAIRY EWES: RUMEN FERMENTATION AND BIOHYDROGENATION OF FATTY ACIDS

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Take home message

Individual variation in the severity of milk fat depression cannot be explained by a single mechanism (e.g., changes in rumen biohydrogenation or milk concentration of antilipogenic fatty acids) but most probably by a complex combination of factors.

In a previous study (Frutos et al., 2017), we were not able to demonstrate that differences in the **milk concentration of some fatty acids** (FA), particularly antilipogenic FA, and in **transcript abundances of candidate genes** involved in mammary lipogenesis explained individual variations in fish oil-induced MFD severity.

It is known that MFD is related to active biohydrogenation intermediates that are produced under certain **feeding conditions that alter rumen function**. Therefore, hypothesis → Differences in the processes of ruminal fermentation and biohydrogenation (BH) of unsaturated FA would account for the individual response in the susceptibility to the low-fat milk syndrome.

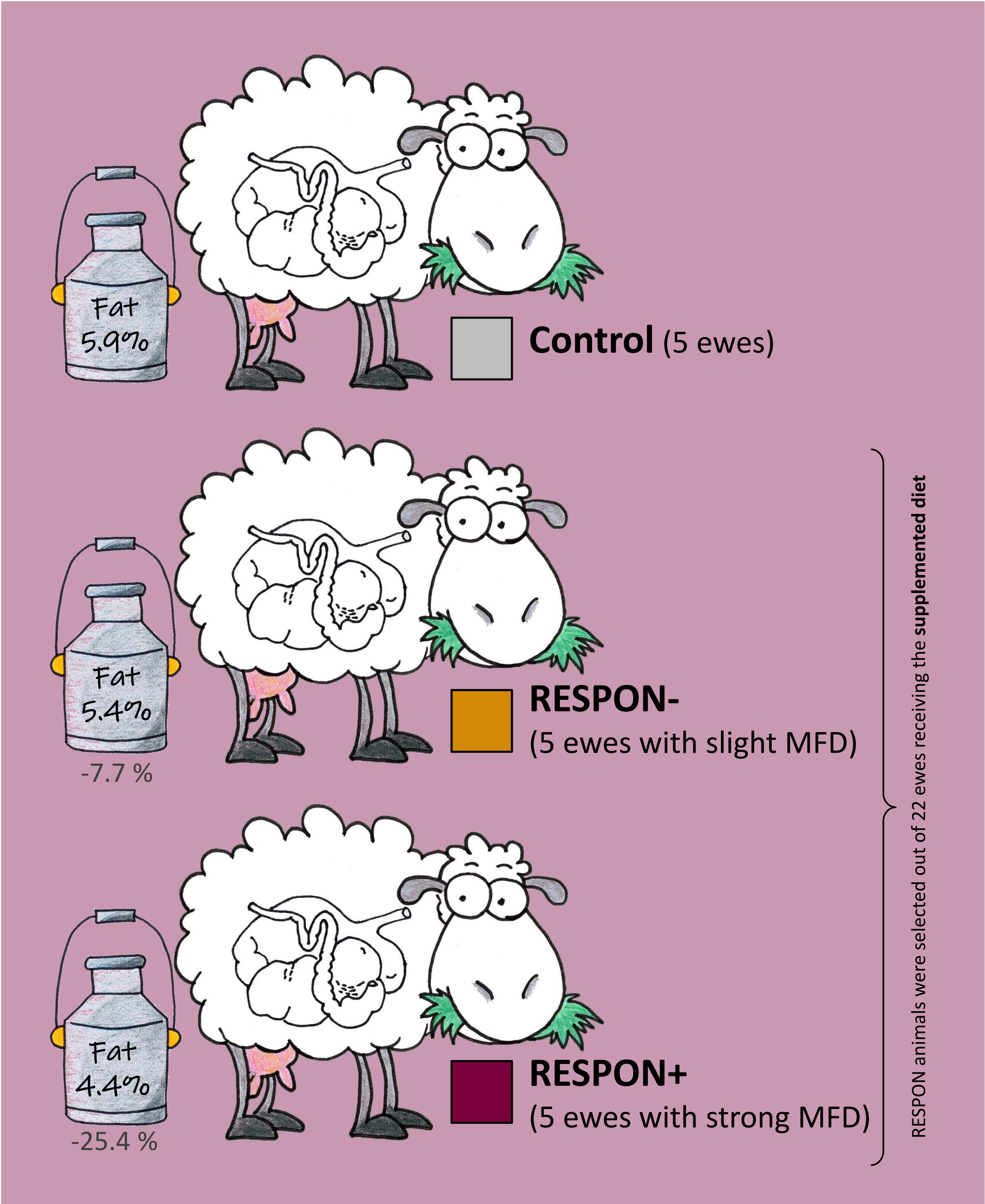
INTRODUCTION

Dairy ewes fed diets supplemented with marine lipids show **large individual variation** in the extent of **milk fat depression** (MFD) but reasons behind this variability are still uncertain.

MATERIAL AND METHODS

Experimental diets (TMR 50:50). 5 weeks

- **Control** diet (no lipid supplementation)
- Supplemented** with 20 g of **fish oil**/kg DM



Rumen fluid samples (36 days on diets; stomach tube)

- **pH**
- **Ammonia** (by UV/Vis)
- **VFA** (by GC)
- **FA profile** (by GC)

CONCLUSION

Rumen concentrations of VFA that are precursors of milk FA, mainly acetate, show lower values in animals with a strong MFD (i.e., in RESPON+). Quite the opposite, changes in rumen BH metabolites show very little variation in ewes displaying different degrees of the syndrome, which would rule out their role as main responsible for MFD severity.

The individual variation in the extent of diet-induced MFD in dairy ewes will probably rely on a complex combination of factors.

RESULTS

Expected differences due to fish oil supplementation

Despite acetate is the main substrate for *de novo* synthesis of FA in dairy ruminants, it was widely accepted that its supply did not affect milk fat yield (Bauman & Griinari, 2001). However, this has been recently challenged by Urrutia and Harvatine (2017) and would merit further research.

	Control	RESPON-	RESPON+	Control vs. RESPON-	RESPON- vs. RESPON+
Ammonia (mg/L)	134	165	182	**	ns
Total VFA (mmol/L)	117	107	92	*	*
Acetate	74.1	67.5	57.6	*	*
Propionate	22.7	21.4	17.9	*	*
Butyrate	16.1	14.3	13.0	*	ns
FA (g/100 g of total FA)					
c6+7 16:1	0.12	0.37	0.49	***	*
c9 16:1	0.10	0.65	0.77	***	ns
17:0 anteiso	0.45	0.40	0.47	ns	**
18:0	46.7	7.56	6.38	***	ns
10-oxo-18:0	<0.01	2.04	2.06	***	ns
c9 18:1	4.36	6.01	6.90	***	ns
c11 18:1	0.55	1.72	1.73	***	ns
t11 18:1	3.11	20.8	20.9	***	ns
t11c15 + t10c15 18:2	0.18	0.80	0.89	***	ns
t10c12 CLA	0.01	0.06	0.04	**	ns
22:6n-3	-	0.83	1.27	-	ns

Only few minor FA differed between RESPON- and RESPON+

Most demonstrated or putative antilipogenic FA showed a higher concentration in ewes fed the fish oil-supplemented diet. On the contrary, surprisingly, none of them differed significantly in relation to MFD intensity (i.e., between RESPON- and RESPON+)

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